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86 Thermostable luciferase of a firefly, gene of a thermostable luciferase of a firefly, novel recombinant DNA, and process for the preparation of a thermostable luciferase of a firefly.

87 The present invention relates to a thermostable luciferase of a firefly wherein an amino acid at the 217-position of the amino acid sequence of the wild-type firefly luciferase or an amino acid equivalent to the amino acid at the 217-position of the luciferase of GENJI firefly or HEIKE firefly is converted into a hydrophobic amino acid, a gene encoding said thermostable luciferase, a vector comprising the gene encoding said thermostable luciferase inserted therein, and a process for the preparation of a thermostable firefly luciferase comprising the use of said vector.

EP 0 524 448 A1

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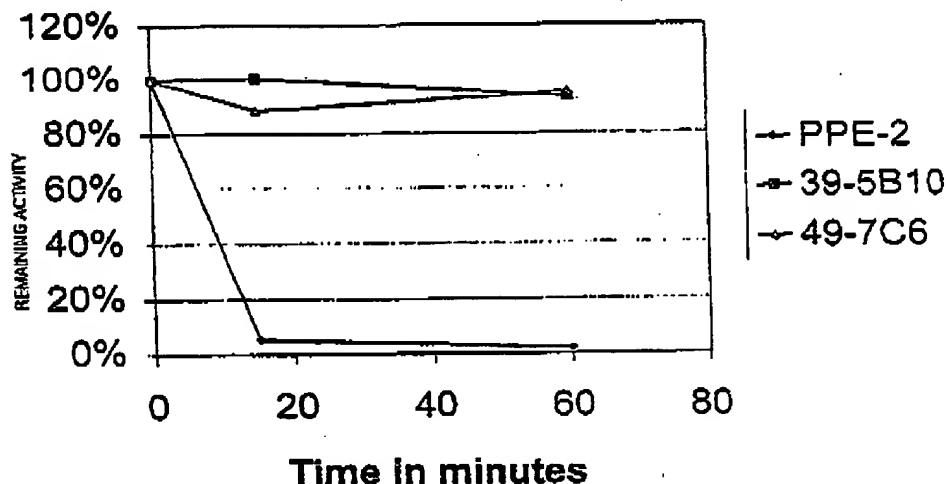
**PCT**P1247/000 PCT SEARCH REPORT  
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1

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: THERMOSTABLE LUCIFERASES AND METHODS OF PRODUCTION

**Stability at 37C normalized to t=0**

## (57) Abstract

Luciferase enzymes with greatly increased thermostability, e.g., at least half lives of 2 hours at 50 °C, cDNAs encoding the novel luciferases, and hosts transformed to express the luciferases, are disclosed. Methods of producing the luciferases include recursive mutagenesis. The luciferases are used in conventional methods, some employing kits.

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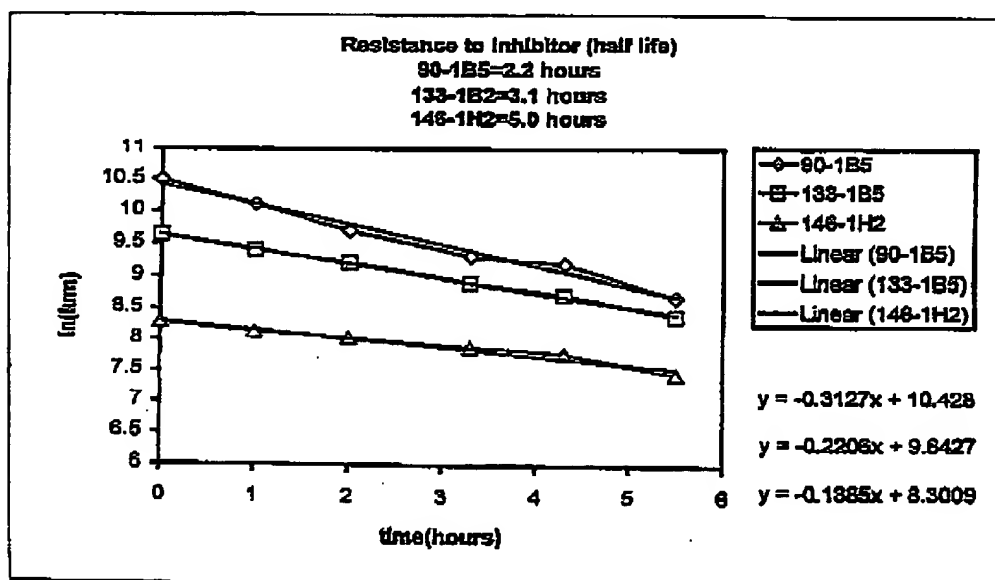
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ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: THERMOSTABLE LUCIFERASES FROM PHOTURIS PENNSYLVANICA AND PYROPHORUS PLAGIOPHTHA-  
LAMUS AND METHODS OF PRODUCTION



(57) Abstract: Luciferase enzymes with greatly increased thermostability, e.g., at least half lives of 2 hours at 50 °C, cDNAs en-  
coding the novel luciferases, and hosts transformed to express the luciferases, are disclosed. Methods of producing the luciferases  
include recursive mutagenesis. The luciferases are used in conventional methods, some employing kits.

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: <b>PCT/GB99/03538</b></p> <p>(22) International Filing Date: <b>26 October 1999 (26.10.99)</b></p> <p>(30) Priority Data: <b>9823468.5</b> <b>28 October 1998 (28.10.98)</b> <b>GB</b></p> <p>(71) Applicant (for all designated States except US): <b>THE SECRETARY OF STATE FOR DEFENCE [GB/GB]; Defence Evaluation and Research Agency, Ively Road, Farnborough, Hampshire GU14 0XL (GB).</b></p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): <b>SQUIRELL, David, James [GB/GB]; CBD, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). MURPHY, Malenie, Jane [GB/GB]; CBD, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). PRICE, Rachel, Louise [GB/GB]; CBD, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). LOWE, Christopher, Robin [GB/GB]; Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 4AT (GB). WHITE, Peter, John [GB/GB]; The Babraham Institute, Babraham Hall, Village of Babraham, Cambridge CB2 4AT (GB). TISI, Laurence, Carlo [GB/GB]; Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 4AT (GB). MURRAY, James, Augustus, Henry</b></p>	<p>[GB/GB]; Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 4AT (GB).</p> <p>(74) Agent: <b>BOWDERY, A., O.; D/IPR, Formalities Section, Poplar 2, MOD Abbey Wood # 19, Bristol BS34 8JH (GB).</b></p> <p>(81) Designated States: <b>AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW. ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</b></p> <p>Published Without international search report and to be republished upon receipt of that report.</p>	
(54) Title: <b>NOVEL ENZYME</b>		
(57) Abstract		
<p>A protein having luciferase activity and at least 60 % similarity to luciferase from <i>Photinus pyralis</i>, <i>Luciola mingrelica</i>, <i>Luciola cruciata</i> or <i>Luciola lateralis</i>, <i>Hotaria paroula</i>, <i>Pyrophorus plagiophtalamus</i>, <i>Lampyrus noctiluca</i>, <i>Pyrocoelia nayako</i> or <i>Photinus pennsylvanica</i> wherein in the sequence of the enzyme, at least one of (a) the amino acid residue corresponding to residue 214 in <i>Photinus pyralis</i> luciferase; (b) the amino acid residue corresponding to residue 232 in <i>Photinus pyralis</i> luciferase; (c) the amino acid residue corresponding to residue 295 in <i>Photinus pyralis</i> luciferase; (d) the amino acid residue corresponding to acid 14 of <i>Photinus pyralis</i> luciferase; (e) the amino acid residue corresponding to amino acid 35 of <i>Photinus pyralis</i> luciferase; (f) the amino acid residue corresponding to amino acid residue 105 of <i>Photinus pyralis</i> luciferase; (g) the amino acid residue corresponding to amino acid residue 234 of <i>Photinus pyralis</i> luciferase; (h) the amino acid residue corresponding to amino acid residue 420 of <i>Photinus pyralis</i> luciferase; (i) the amino acid residue corresponding to amino acid residue 310 of <i>Photinus pyralis</i> luciferase: is different to the amino acid which appears in the corresponding wild type sequence and wherein the luciferase enzyme has increased thermostability as compared to an enzyme having the amino acid of the corresponding wild-type luciferase at this position.</p>		

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(54) Title: MUTANT LUCIFERASE

(57) Abstract: A recombinant protein having luciferase activity and at least 60 % similarity to a wild-type luciferase wherein in the sequence of the enzyme, the amino acid residue corresponding to residue 357 in *Photinus pyralis* luciferase is mutated as compared to the corresponding wild-type luciferase, such that the luciferase enzyme is able to emit light at a different wavelength as compared to the corresponding wild-type luciferase and/or has enhanced thermostability as compared to the corresponding wild-type luciferase. In general, the residue corresponding to 357 in *Photinus pyralis* luciferase is changed from an acidic amino acid to a non-acidic amino acid and preferably an uncharged polar amino acid such as tyrosine. Mutant luciferases in accordance with the invention can produce a large (50nm) wavelength shift in emitted light and have good thermostability. The resultant colour shift can be reversed by addition of coenzyme A. These properties make the mutant particularly useful in a variety of assays.